A two-toed population of the critically endangered Retro Slider Skink, *Lerista allanae* (Longman, 1937) (Reptilia: Scincidae)

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ABSTRACT

Surveys to date for the recently re-discovered Retro Slider, *Lerista allanae*, show it to have a narrow, highly restricted distribution. There are currently no populations known to occur in protected areas, with the majority of recent records coming from roadside verges. There is considerable variation in colour pattern and morphology which had not been captured in earlier descriptions based on smaller sample sizes. Colour pattern is commonly grey to tan but some individuals can be very dark to almost black. Additionally, one population possesses two clawed digits on the hind foot, rather than one as seen in all previously examined specimens. While this variation could suggest the presence of cryptic species, a genetic assessment shows it to be intra-specific. The species remains of high conservation significance despite an increase in its Extent of Occurrence.
❒ *Conservation, central Queensland, Brigalow Belt, intra-specific variation*

The Retro Slider Skink, *Lerista allanae*, is a species of special interest, which until recently was only known from 13 museum specimens, with most collected in the 1930s. They all came from a small area in the Clermont region of Queensland's Central Brigalow Belt Northern Bioregion. Covacevich *et al.* (1996) reviewed the registration details associated with these specimens and information relating to their collection contained in archival correspondence. These authors also conducted field surveys on Retro Station, the type locality for this species, and surrounding properties. They noted broad scale changes in land use between 1929 (year of first collection) and 1996 which led them to surmise that *L. allanae* was probably extinct due to the broad scale clearing and degradation of its habitat. In consequence, the species has been listed as Endangered under state and national legislation and Critically Endangered by the IUCN (Australasian Reptile & Amphibian Specialist Group 1996; Australian Government 1999; Queensland Government 1992).

The species was rediscovered in September 2009 when a dead specimen was found in a swimming pool skimmer box on a property near Clermont. This was sent to the Queensland Museum (specimen QMJ88031) where its identification was confirmed. Follow-up surveys were undertaken to assess the status and extent of the remaining population. The surveys broadened the Area of Occupancy and revealed new information on colour pattern and limb morphology which is reported herein.

METHODS

Surveys conducted since September 2009 have resulted in the capture of a further 20 *L. allanae* specimens. All but one of the specimens were obtained by systematically raking leaf-litter and the underlying friable surface soils beneath trees, shrubs and dead grass mats. A single individual was captured in a pit trap with a short drift fence. Handling of those captured was kept to a minimum. They were weighed using a Pesola spring balance. The snout-vent length and total length was measured using electronic Vernier callipers. Location information was captured using hand-held Garmin 76CSx GPS units. Two specimens were vouchered and lodged at the Queensland Museum (QMJ88428 and QMJ94046). Of those not vouchered, most were photographed and tail tipped (tissue for genetic analyses) then released at their capture site. All survey activities were conducted under DAFF Animal Ethics Committee Approval number SA2013/07/431 and DSITI safety guidelines. Full methods are detailed in Borsboom (2016; 2012), Borsboom *et al.* (2010) and, Borsboom and Ferguson (2015).

Genetics. Couper *et al.* (2016) included two one-toed specimens of *L. allanae* in their recent study of the *Lerista wilkinsi* complex. The current study expanded that dataset by adding eleven new samples, including tissues from both one-toed (n = 3, Queensland Museum collection) and two-toed (n = 8, this study) populations for a total of thirteen specimens. DNA extraction, amplification (including loci targeted: mitochondrial 12S and 16S ribosomal rRNA (12S, 16S) and NADH dehydrogenase subunit 4 (ND4) including adjacent transfer tRNA fragments tRNA-His, tRNA-Ser and tRNA-Leu (tRNAs) and the nuclear ATP synthetase-β subunit intron) and sequencing methods, alignment and sequence evolution model choice are as described in Couper *et al.* (2016). All new *L. allanae* sequences were incorporated into the existing alignment generated for Couper *et al.* (2016) and have been deposited in GenBank nucleotide sequence database (see Appendix for details).

Analyses were expanded from Couper *et al.* (2016) with relationships among the two *L. allanae* morphological variants investigated using two different model-based phylogenetic analyses. These were performed via XSEDE (version 3.2.3.) on the CIPRES Science Gateway version 3.3 (Miller *et al.* 2010) using RAxML v8.2.9 (Stamatakis 2014) for maximum likelihood analysis and MrBayes v3.2.6 (Ronquist *et al.* 2012) for Bayesian inference. Both analyses were implemented on the total concatenated dataset incorporating the partitioning strategy found by Skinner (2010) to provide the best fit for the data (7 partitions: 12S, 16S, ND4 first, second and third codon positions, flanking tRNAs and nucATP).

For the maximum likelihood search in RAxML, 1000 bootstrap pseudo-replicates were performed followed by a search for the best performing tree. For the Bayesian inference analyses, we used settings with a Markov chain Monte Carlo of 10 000 000 generations, with the chain sampled every 1000 generations. Burn-in values for each run were set at 2 500 000 generations (2 500 trees) after empirical values of stabilizing likelihoods and the average standard deviation of the split frequencies reached less than 0.01 indicating that convergence of the MCMC chains had been reached (Posada & Crandall 1998). Maximum clade credibility trees for each run were generated using TreeAnnotator v1.7.5 (as part of the BEAST software package, Drummond *et al.* 2012) with posterior probability limits set for branch support greater than 0.6 (Posada & Crandall 1998).

RESULTS

Distribution. Exact localities will not be disclosed in this paper as the species is highly localised and likely to be sensitive to disturbance (see

Lindenmayer & Scheele 2017 for a discussion of this issue). New records reported in this paper are mapped in relation to historical records in Figure 1.

The type series of *Lerista allanae* was collected in 1936–37 from an unspecified area of Retro Station. At this time, Retro was much larger than its present 2209 ha and parts of 'old Retro' now fall within the boundaries of neighbouring properties (Covacevich *et. al.* 1996). While no *L. allanae* have been found during recent surveys on the current Retro property, the species is present in areas that were excised from Retro since the type description and one of these may well be the site from where the type series was collected. Surveys since September 2009 have found *L. allanae* at four new sites, two with single records only. Two estimates of a species' range size used and defined by the IUCN are Extent of Occurrence (EOO) and Area of Occupancy (AOO) (IUCN 2012). Using the data we have collected for *L. allanae*, these parameters are 100 km^2 for EOO and <10 km^2 for AOO. An AOO of 40.7 ha was derived from 100 m buffers around vouchered records, the AOO adjusted for overlapping buffers. The recent records are associated with clumps of trees and shrubs surrounded by grassland and most of these are along road easements. Consequently, most of the survey effort has focussed on roadside vegetation with the known EOO being largely linear, extending 40 km in a north/south direction between Clermont and Emerald. Although the EOO may increase with further survey effort, it is likely to remain narrow and to have been greatly reduced by extensive land clearing practices from 1940 onwards (Covacevich *et al.* 1996). Preliminary surveys in an east-west direction have, to date, failed to extend the distribution. A specimen in the South Australian Museum, collected prior to November 1948, came from Logan Downs, around 40 km to the north, suggesting that, historically, the distribution was more extensive. It should be noted however that, like Retro Station, Logan Downs was much more extensive in 1948 than its present area so the accuracy of this record is low.

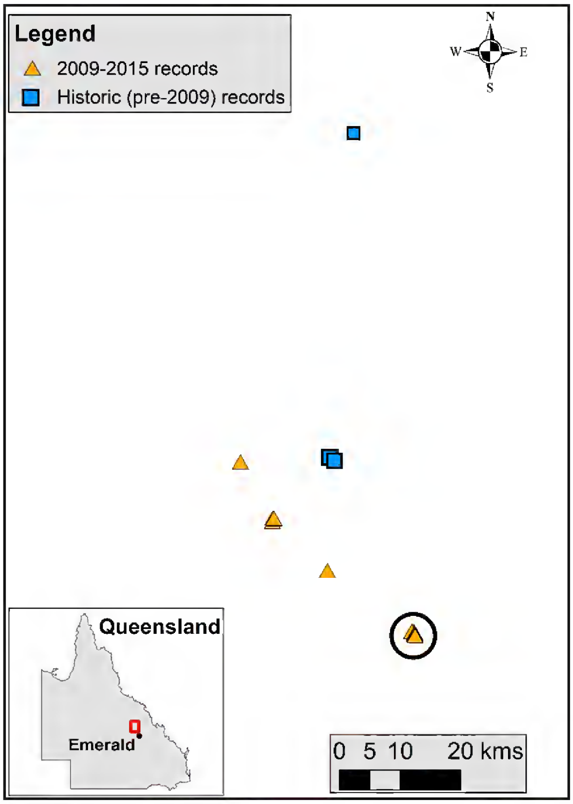

FIG. 1. Map showing new records of *Lerista allanae* obtained in this study in relation to historical records. Two-toed *L. allanae* records are circled.

Toe morphology. The presence of a monodactyl hind limb with a single clawed digit is regarded as diagnostic for *L. allanae* (Fig. 2A; Cogger 2014; Longman 1937). This condition, reported from the small series of specimens then available, is not constant with some individuals encountered during the present study bearing didactyl hind limbs (11 of 36 records; Fig. 2B). This condition may be confined to the most southern population where it was observed in all individuals captured (11/11). Twelve of the 13 specimens held in museum collections come from north of Capella and these all have monodactyl hind limbs. Where a second digit is present, it is small, around 40% the length of the longer digit (in QMJ94046, shown in Fig 2B, a vouchered specimen from the southern population, the second digit is only 0.7 mm

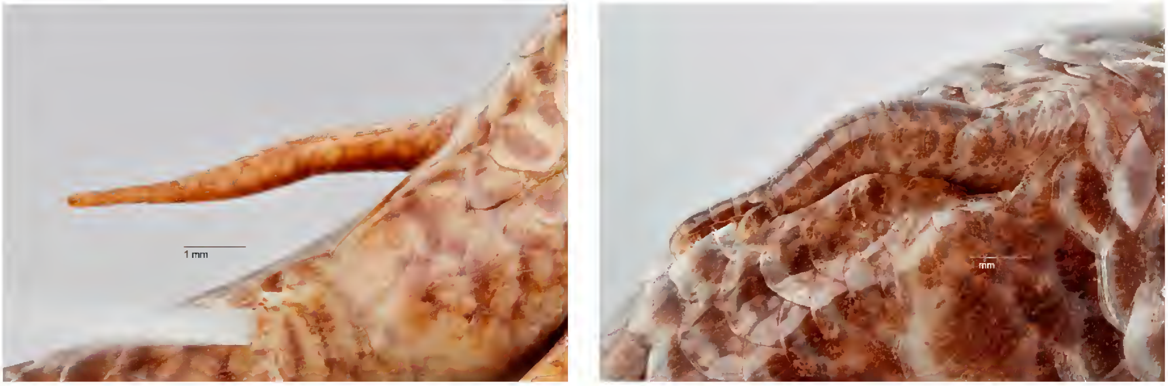

FIG. 2. Toe arrangement of *Lerista allanae*; **A**, the more common single digit (QMJ6430); **B**, two toes (QMJ94046). Photos by G. Thompson.

long and bears three subdigital lamellae). The claw is poorly developed, its tip only just extending beyond the surrounding scales. Consequently, the second digit could be easily overlooked and this character may conceivably have been missed in some of the released specimens from the more northern populations (seven individuals).

Colour Pattern. In life, *L. allanae* is usually coppery-brown above with the dorsal colouration two to three scale rows either side of the vertebral line (this contrasts with Longman's 1937 description of preserved material 'Ridgeway's drab gray'). This colour is particularly rich in juveniles (Fig. 3A) but tends to fade towards tan in larger individuals (Fig. 3B).

Adults. The dorsal pattern generally consists of a series of four or more almost continuous, dark longitudinal lines extending from the nuchals to the base of the tail, with individual scales marked with a dark posterior edge or streak. The flanks are usually lighter, off-white to cream with the posterior half of each scale dark brown to black, producing a heavily spotted or speckled effect which usually extends to the ventral surface (referred to as 'prettily marked with dark spots' in type description, Longman, 1937). In some specimens the ventral scales have finer dark edges giving a more reticulated appearance. Head shields are coppery to tan with dark edging and scattered blotches. Upper labials are strongly pigmented. Tip of snout (rostral, mental, nasals, first supralabial and first infralabial) sometimes with a waxy opaque appearance (this may relate to a stage in skin sloughing and is characteristic of *Lerista*, Greer 1989).

TABLE 1: Average sequence divergence for *Lerista allanae.*

Locus	Within One-toed	Within Two-toed	Between One-toed and Two-toed	Ave among all *Lerista* species
12S	0.6%	0.4%	0.4%	8.61%
16S	0.9%	0.3%	0.3%	6.52%
ND4	1.0%	0.4%	0.2%	13.45%
tRNAs	2.0%	0.0%	0.2%	12.37%
nucATP	0.00%	0.00%	0.00%	3.82%

Juveniles. The pattern is generally more speckled than in adults with the dark dorsal lines less well defined. Most of the juveniles from the south Capella site were coppery brown above with dark edges to the scales giving a reticulated appearance that breaks into dark speckling with increasing size. Ventral surface as for adults although in some individuals the dark reticulations are more extensive giving the appearance of a darker ventral surface.

Melanistic pattern. Some specimens are so strongly pigmented their general appearance is dark brown to black. In QMJ88428 (a juvenile) there is little demarcation between the dorsal,

FIG 3. Colour variability in *Lerista allanae*; **A**, coppery brown pattern most often seen in juveniles (tissue sample QMA13152); **B**, most frequently seen tan colour (tissue sample QMA13156); **C**, the dark colour pattern observed in some southern individuals (QMJ94046). Photos by D. Ferguson.

lateral and ventral colours, the flanks are strongly speckled and the ventral surface is tinged with brown. Specimen QMJ94046 (adult, Fig. 3C) is extremely dark, both above and below, although, with closer scrutiny, dark scale edges, spots and speckles are still discernible.

Genetic assessment. Both maximum likelihood and Bayesian inference phylogenetic analyses returned near identical topologies and clearly support the southern two-toed and northern one-toed populations as a single, monophyletic species with maximum bootstrap (100) and posterior probability (1.00) support respectively (Fig. 4). Furthermore, genetic differences among the two morphotypes are minimal, with average sequence divergence estimates ranging between 0.0–0.4% across all loci (Table 1).

DISCUSSION

Lerista allanae was thought to be extinct but is now known to persist in a small area of central Queensland. The few specimens available prior to its rediscovery in 2009 did not allow a full description of the morphological variability within this species. An ability to confidently identify a species in the field is critical to its management. With more specimens and genetic information, we have a better understanding of intraspecific variability. Morphological variability is proving to be a common feature of *Lerista*. Several Western Australian species have a variable number of digits (for example, the three-toed *L. kalumburu* has a geographically discrete population of two-toed individuals within it; Amey & Worthington Wilmer 2014). However, this phenomenon seems to be less common in eastern Australia, where the hind limb arrangement has been seen as a diagnostic character for separating species, for example, *L. emmotti* from *L. punctatovittata* and *L. allanae* from *L. colliveri* (Couper & Ingram 1992; Ingram *et al.* 1993). While genetic analysis shows these to be valid species (Skinner 2010), it is plain that relying on morphological evidence alone to diagnose species of *Lerista* can, at times, be unsatisfactory.

While some variability of colour pattern is common within species of *Lerista*, it normally consists of variation in the intensity of stripes, from indistinct rows of spots to clearly defined stripes. The extremes observed within the small population of *L. allanae* have not been documented in any other species. The significance of this variability is unclear.

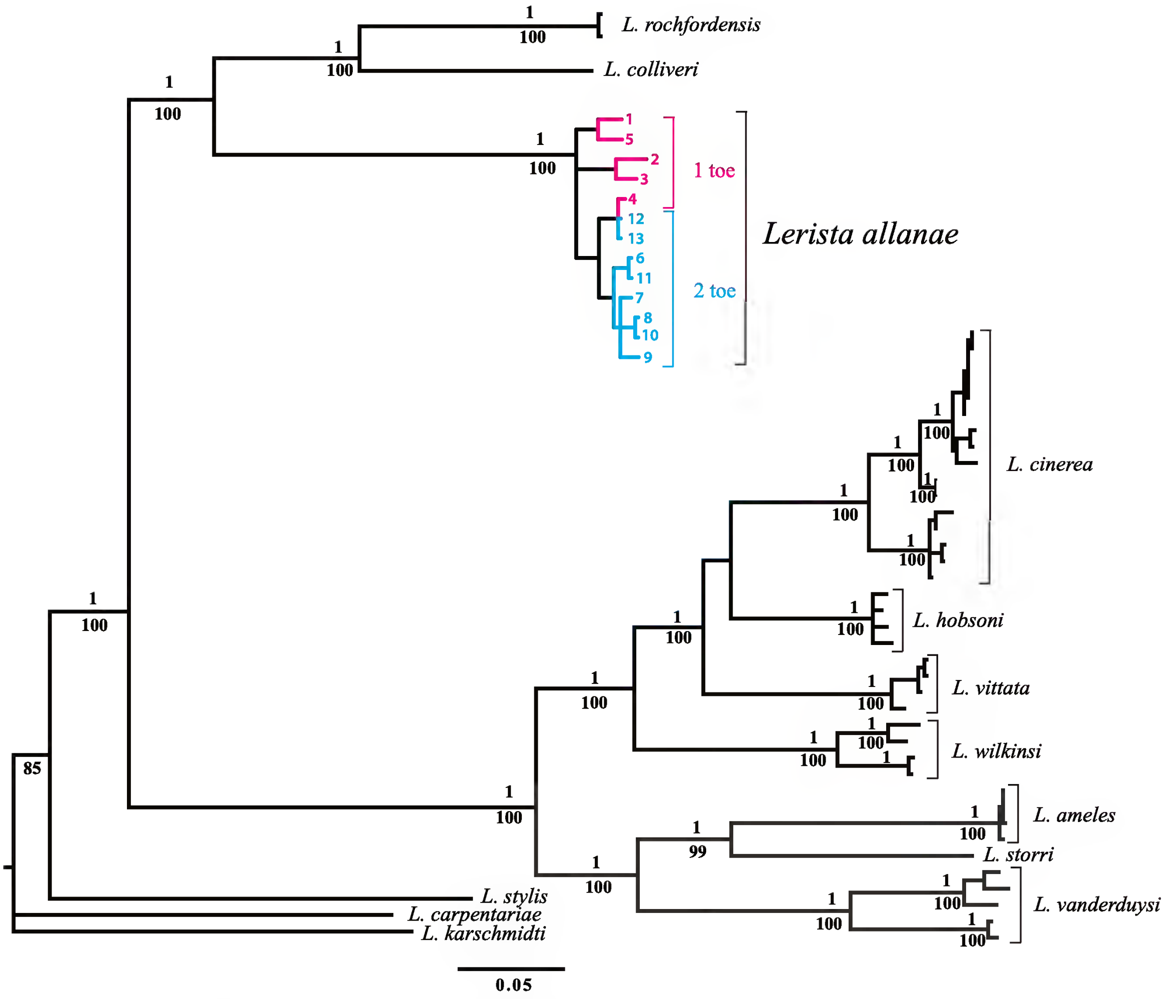

FIG 4. Maximum-likelihood tree based on the full concatenated dataset. Clade support is indicated by bootstrap values ≥ 70 below the branch and posterior probabilities ≥ 0.95 representing a topologically identical maximum clade credibility Bayesian tree are above the branch. Scale represents average number of substitutions per site.

The surveys described herein have increased the known EOO for *L. allanae* but the species remains highly localised in a modified landscape with additional disturbance potentially elevating its risk of extinction. The AOO is entirely outside of protected areas and is confined to a narrow strip adjacent to areas of intense agricultural activity on one side and a main road on the other. Work on other *Lerista* species in north Queensland shows they have naturally fragmented distributions, being isolated in small patches of suitable habitat, i.e., friable soil that they can 'swim' through (Couper *et al.* 2016). This makes them vulnerable to disturbance as a reasonably small-scale development has the potential to eliminate entire populations and their limited dispersal ability makes recolonization of disturbed areas unlikely. Almost the entire known range of *L. allanae* is now confined to roadside easements, areas that are potentially impacted by road and rail works, invasive weeds, weed control, grazing, fire and the activities of reptile enthusiasts keen to tick another species off their 'twitcher' lists.

Lerista allanae is a cryptic, fossorial species that requires intensive survey effort to uncover. This is why, despite the survey effort that has occurred since its rediscovery, the full distribution of *L. allanae* remains largely unknown. The record from Logan Downs (registered in 1948) is furthest from the new records, suggesting a wider former distribution but the historical distribution must remain speculative.

The most effective survey technique for *L. allanae* to date, raking through leaf litter and the subsoil environment, involves disruption to a sensitive, very limited microhabitat. Because of this potential damage, future searches for *L. allanae* should only be undertaken by approved personnel in attempts to extend its distribution or to confirm its continued existence at known localities following habitat disturbance or other causes for concern. While surveys without clear, defensible conservation goals must be avoided, surveys of suitable habitat on surrounding properties are recommended in order to locate additional populations. *Lerista allanae* remains one of Australia's most critically endangered reptiles.

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APPENDIX

Tissue registration and GenBank sequence numbers for material examined in this study.

Species	Tissue Number	12S rRNA	16S rRNA	ND4+tRNAs	ATP
Lerista allanae - Capella 1	QM A005540	KU309145	KU309187	KU309272	KU309229
Lerista allanae - Capella 2	QM A006515	KU309146	KU309188	KU309273	KU309230
Lerista allanae - Capella 3	QM A006531	MF959776	MF959787	MF959798	MF959808
Lerista allanae - Capella 4	QM A009274	MF959777	MF959788	N/A	MF959809
Lerista allanae - Capella 5	QM A009277	MF959778	MF959789	MF959799	MF959810
Lerista allanae - Capella 6	QM A013150	MF959779	MF959790	MF959800	MF959811
Lerista allanae - Capella 7	QM A013151	MF959780	MF959791	MF959801	MF959812
Lerista allanae - Capella 8	QM A013153	MF959781	MF959792	MF959802	MF959813
Lerista allanae - Capella 9	QM A013154	MF959782	MF959793	MF959803	MF959814
Lerista allanae - Capella 10	QM A013155	MF959783	MF959794	MF959804	MF959815
Lerista allanae - Capella 11	QM A013156	MF959784	MF959795	MF959805	MF959816
Lerista allanae - Capella 12	QM A013157	MF959785	MF959796	MF959806	MF959817
Lerista allanae - Capella 13	QM A013158	MF959786	MF959797	MF959807	MF959818
Lerista ameles - Mt Surprise 1	ABTC 77124	KU309147	KU309189	KU309274	KU309231
Lerista ameles - Mt Surprise 2	ABTC 77125	KU309148	KU309190	KU309275	KU309232
Lerista ameles - Mt Surprise 3	ABTC 77144	KU309149	KU309191	KU309276	KU309233
Lerista ameles - Mt Surprise 4	ABTC 77171	KU309150	KU309192	KU309277	KU309234
Lerista cinerea - Warrawee Station 1	QM A013421	KU309151	KU309193	KU309278	KU309235
Lerista cinerea - Warrawee Station 2	QM A013422	KU309152	KU309194	KU309279	KU309236

Appendix continued …

Species	Tissue Number	12S rRNA	16S rRNA	ND4+tRNAs	ATP
Lerista cinerea - Warrawee Station 3	QM A013423	KU309153	KU309195	KU309280	KU309237
Lerista cinerea - Warrawee Station 4	QM A013424	KU309154	KU309196	KU309281	KU309238
Lerista cinerea - Warrawee Station 5	QM A013425	KU309155	KU309197	KU309282	KU309239
Lerista cinerea - Warrawee Station 6	QM A013426	KU309156	KU309198	KU309283	KU309240
Lerista cinerea - Bletchington Park	QM A006562	KU309157	KU309199	KU309284	KU309241
Lerista cinerea - Gregory Development Rd	QM A013451	KU309158	KU309200	KU309285	KU309242
Lerista cinerea - Rishton Scrub 1	QM A013427	KU309159	KU309201	KU309286	KU309243
Lerista cinerea - Rishton Scrub 2	QM A013428	KU309160	KU309202	KU309287	KU309244
Lerista cinerea - Rishton Scrub 3	QM A013429	KU309161	KU309203	KU309288	KU309245
Lerista cinerea - Sellheim Scrub 1	QM A013430	KU309162	KU309204	KU309289	KU309246
Lerista cinerea - Sellheim Scrub 2	QM A013431	KU309163	KU309205	KU309290	KU309247
Lerista cinerea - Sellheim Scrub 3	QM A013432	KU309164	KU309206	KU309291	KU309248
Lerista cinerea - Sellheim Scrub 4	QM A013433	KU309165	KU309207	KU309292	KU309249
Lerista cinerea - Sellheim Scrub 5	QM A013434	KU309166	KU309208	KU309293	KU309250
Lerista colliveri	QM A003450	KU309167	KU309209	KU309294	KU309251
Lerista hobsoni - Lolworth Homestead 1	ABTC 72912	KU309168	KU309210	N/A	KU309252
Lerista hobsoni - Lolworth Homestead 2	ABTC 72913	KU309169	KU309211	KU309295	KU309253
Lerista hobsoni - Lolworth Homestead 3	ABTC 72914	KU309170	KU309212	KU309296	KU309254
Lerista hobsoni - Pentland	QM A013450	KU309171	KU309213	KU309297	KU309255
Lerista rochfordensis - Barrabas Scrub 1	QM A013440	MF589181	MF589191	MF589212	MF589202
Lerista rochfordensis - Barrabas Scrub 2	QM A013441	MF589182	MF589192	MF589213	MF589203
Lerista rochfordensis - Barrabas Scrub 3	QM A013442	MF589183	MF589193	MF589214	MF589204
Lerista rochfordensis - Barrabas Scrub 4	QM A013443	N/A	MF589194	MF589215	MF589205

Appendix continued …

Species	Tissue Number	12S rRNA	16S rRNA	ND4+tRNAs	ATP
Lerista rochfordensis - Barrabas Scrub 5	QM A013444	MF589184	MF589195	MF589216	MF589206
Lerista rochfordensis - Barrabas Scrub 6	QM A013445	MF589185	MF589196	MF589217	N/A
Lerista rochfordensis - Barrabas Scrub 7	QM A013446	MF589186	MF589197	MF589218	MF589207
Lerista rochfordensis - Barrabas Scrub 8	QM A013447	MF589187	MF589198	MF589219	MF589208
Lerista rochfordensis - Rochford Scrub 1	QM A013435	KU309172	KU309214	KU309298	KU309256
Lerista rochfordensis - Rochford Scrub 2	QM A013436	KU309173	KU309215	KU309299	KU309257
Lerista rochfordensis - Rochford Scrub 3	QM A013437	MF589188	MF589199	MF589220	MF589209
Lerista rochfordensis - Rochford Scrub 4	QM A013438	MF589189	MF589200	MF589221	MF589210
Lerista rochfordensis - Rochford Scrub 5	QM A013439	MF589190	MF589201	MF589222	MF589211
Lerista storri - Almaden	QM A005045	KU309174	KU309216	KU309300	KU309258
Lerista vanderduysi - Blackbraes 1	QM A000418	KU309175	KU309217	KU309301	KU309259
Lerista vanderduysi - Blackbraes 2	QM A001142	KU309176	KU309218	KU309302	KU309260
Lerista vanderduysi - Blackbraes 3	QM A002098	KU309177	N/A	N/A	KU309261
Lerista vanderduysi - Gilbert Station 1	QM A008448	KU309178	KU309219	N/A	KU309262
Lerista vanderduysi - Gilbert Station 2	QM A008462	N/A	KU309220	N/A	KU309263
Lerista vittata - Mt Cooper Station 1	QM A013417	KU309179	KU309221	KU309303	KU309264
Lerista vittata - Mt Cooper Station 2	QM A013418	KU309180	KU309222	KU309304	KU309265
Lerista vittata - Mt Cooper Station 3	QM A013419	KU309181	KU309223	KU309305	KU309266
Lerista vittata - Mt Cooper Station 4	QM A013420	KU309182	KU309224	KU309306	KU309267
Lerista wilkinsi - Torrens Creek 1	ABTC 76998	KU309183	KU309225	KU309307	KU309268
Lerista wilkinsi - Torrens Creek 2	ABTC 121865	KU309184	KU309226	KU309308	KU309269
Lerista wilkinsi - Torrens Creek 3	QM A013448	KU309185	KU309227	KU309309	KU309270
Lerista wilkinsi - Torrens Creek 4	QM A013449	KU309186	KU309228	KU309310	KU309271

Appendix continued …

Species	**Tissue Number**	**12S rRNA**	**16S rRNA**	**ND4+tRNAs**	**ATP**
Outgroups					
Lerista carpentariae	N/A	EF672763	EF672834	EF672975	EF672905
Lerista karlschmidti	N/A	EF672787	EF672858	EF672999	EF672929
Lerista stylis	N/A	EF672811	EF672882	EF373023	EF672952

Abbreviations

ABTC = Australian Biological Tissue Collection

QM = Queensland Museum